Serological Study of Typhoid Fever among Patients Attending Nasarawa State University Clinic Keffi, Nigeria

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ABSTRACT

Typhoid fever causes major health problems especially in low- and middle-income individuals. A serological study of typhoid fever was carried out among 150 patients attending Nasarawa State University clinic Keffi, Nasarawa State, Nigeria from April to June 2019. Blood samples were collected for Widal test, blood culture and Enzyme-linked immunosorbent assay (ELISA) test. Patients aged between 5 to 60 who reported to the clinic with fever (temperature 37.6 °C to 42 °C) were enlisted for the study. A total of 81 (54.0%) of the study participants were females while 69 (46.0%) were males. Out of the 150 patients, 63 (42%) were positive with typhoid fever by Widal test, 51 (34%) by blood culture and 82 (54.6%) by ELISA. The sensitivity and specificity values for Widal test were 84% and 88.2% while Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 84% and 82.3% compared to (gold standard) ELISA (100%), whereas the sensitivity and specificity values for blood culture was 68.0% and 64.7% while PPV and NPV was 62.1% and 64.7% compared to (gold standard) ELISA (100%). Data were analyzed using Chi-square ($\chi^2$) to determine the relationship between the variables used, there was a significant relationship between age and gender of the patients, and also, there was a significant relationship between the patients and their respective occupation by Widal test. Blood culture test revealed significant relationship between age and gender, while on the occupation of the patients, there was no co-relationship. A two-by-two ($2 \times 2$) diagnostic was used to determine sensitivity and specificity, PPV and NPV of the diagnostic techniques used. ELISA technique of diagnosis showed higher accuracy than the other techniques used in the study. The findings of this study showed the need for safe portable water to avoid drinking contaminated water in the affected community.

Keywords: ELISA, patients, serology, typhoid fever.

1. Introduction

A global estimate of typhoid is said to have caused 21.7 million illnesses and 217,000 fatalities annually [1]. An estimated illness of 21.6 million and 216,510 deaths were reported globally in the year 2000 which was in contrast to the previous estimate of 16 million illnesses and 600,000 deaths recorded 16 years ago. The increase in typhoid fever's global burden might have been due to the increase in the global population by 20% from 4.8 billion to 6.1 billion. However, advancements in different diagnostics might have played a role. On the contrary, the incident rates of typhoid fever in some parts of the world have declined over the past several decades [2].

In Nigeria, typhoid fever prevalence rates were put at 42% in Owerri [3], 80.1% in Abeokuta among patients with febrile illness [4], and 81.5% in Minna [5], [6]. Although Nigeria is one of Africa's most densely populated countries with large urban development areas, surprisingly, little reliable data are available on microbial culture of the etiologic agents in children or adults. This challenges data comparison with other regions, including other sub-Saharan African countries where such data are available [7]. Due to the scarcity of data on typhoid incidence in Africa,
WHO requested more epidemiological data to ascertain the typhoid fever burden in Sub-Saharan Africa. Typhoid fever (enteric fever) is a systemic prolonged feverish illness caused by certain Salmonella serotypes. Salmonella enteric serotype typhi (S. typhi) and Salmonella enteric serotype paratyphi (S. paratyphi A, S. paratyphi B, and S. paratyphi C) are the species that cause typhoid fever. S. typhi is the most common serotype of Salmonella that causes typhoid fever [8]. Poor disposal of human excreta, poorly equipped latrines with water facilities, poor hand washing habits, and untreated water usage are the main causes of transmission of typhoid fever in developing countries [9]. So much so that serological assays such as Widal test are less than optimal. However, this form of diagnosis is the most readily available test for typhoid diagnosis in rural settlements and areas with poor health infrastructure [10], and the most important of these methods are blood culture and DNA detection tests, which are sensitive and have high specificity [11]. This provides the most conclusive confirmation of enteric fever since the Enzyme-linked immunosorbent assay (ELISA) technique is a method that detects the immune response of the body, determining the presence of antibodies in the acute and chronic phases [12] and given that ELISA test uses components of the system such as IgG, IgA, IgM antibodies. Therefore, this study aims to determine cases of typhoid fever and establish a safe and reliable tool that yields acceptable results.

2. Materials and Methods

2.1. Study Site

This study was carried out in Nasarawa State, Keffi Local Government Area. Nasarawa State is located in the North Central part of Nigeria within the Guinea savannah vegetation zone (see Fig. 1). It shares borders with Plateau, Benue, and Kaduna states, as well as the federal capital territory of Abuja. Keffi lies between latitude 7°45′ and 9°25′ of the equator and longitude 7° and 9°37′E of the Greenwich Meridian. It has a moderate annual rainfall of about 111.75 mm with a temperature range between 29 °C and 35 °C. Keffi Local Government according to the National Population Commission of Nigeria [13] has an estimated population of 92,550. Keffi shares a boundary with three Local Government Councils, namely Kokona Local Government Council to the East, Nasarawa Local Government Council to the West, and Karu Local GC to the North.

2.2. Studied Parameters and Data Collection

A cross-sectional study was conducted among 150 patients to collect both qualitative and quantitative data on patients aged 5 to 60 with temperature readings above 37.5 °C at the laboratory section of the Nasarawa State University clinic Keffi between the months of March and June 2019. Blood samples were collected from patients suspected to have typhoid fever to determine the prevalence of typhoid fever in relation to some socio-demographic characteristics.

2.3. Sample Size Determination

The formula below was used to determine the sample size.

\[
Sample \ size = \frac{(Z_{1-\alpha/2})^2 SD^2}{d^2}
\]

\[
Z_{1-\alpha/2} = \text{Standard normal variate (at 5% type I error (p < 0.05) it is 1.96),}
\]

\[
SD = \text{Standard deviation of variable,}
\]

\[
d = \text{Absolute error or precision—to be decided by the researcher.}
\]

\[
Z_{1-\alpha/2} = 1.96, \quad SD = 31.2, \quad d = 5.
\]

Therefore:

\[
Sample \ size = \frac{1.96^2 (31.2)^2}{5^2} = 149.5 \approx 150
\]

2.4. Test Procedure

2.4.1. Widal Test

An Omega Widal test kit containing reagent O and H antigen was used in the study to detect Salmonella spp in serum samples in accordance with Cheesbrough’s method [14]. Serum samples were separated from whole blood, which was collected in an Ethylene Diamine Tetra Acetic Acid (EDTA) sample bottle. A drop of the patient’s serum was pipetted on a slide, and a drop of respective Widal reagents was pipetted on the serum. Each content was mixed and then rocked gently. After 2 minutes, the result was read based on the presence of visible agglutination. The degree of agglutination was recorded in liters as 1:40, 1:80, 1:160, and 1:320.

2.4.2. Blood Culture Test

Whole blood samples were cultured using Brilliant Green Bile Broth (BGBB) and Selenite F Broth for enrichment before plating the samples in SS (Salmonella-Shigella) agar and Xylose Lysine Deoxycholate (XLD) Agar according to the methods of [14] and [15]. Culturing was done manually according to the manufacturer’s instructions by inoculating into Brilliant Green Bile Broth and incubated aerobically at 35 °C–37 °C for 7 days and examined visually daily for evidence of bacterial growth. Indicators of bacterial growth that were used include turbidity of the blood-broth mixture, growth of microcolonies, hemolysis, color changes, and gas production. After 24 hours of incubation, all cultures showing growth or no growth were sub-cultured onto solid media plates of MacConkey agar (MAC), Blood agar (BA), and Chocolate agar (CA). The BA and MAC agar were incubated at 35 °C–37 °C aerobically and CA anaerobically (5%–10% CO₂) for 24–48 hours. In the case where Brilliant Green Bile Broth showed no growth up to day 7, subcultures were repeated from the broth on day 7 before it was discarded. On MacConkey, Salmonellae are non–lactose fermenters and form moist colonies of blood agar.

2.4.3. Salmonella Typhi ELISA IgG Test

To determine the IgG of Salmonella typhi, an ELISA kit manufactured by MyBioSource USA with catalog number MBS494612 (96T) procedure was followed according to the company’s instructions.
2.5. Data Analysis

Data were entered and arranged in Statistical Package for Social Sciences (SPSS) v22 (Chicago, USA). Chi-square ($\chi^2$) was used to determine if the relationship between the testing technique and Salmonella typhi was significant. A $2 \times 2$ diagnostic table was used to calculate sensitivity, specificity, and negative and positive predictive values for the widal and blood culture tests using ELISA as the gold standard.

3. Results

3.1. Prevalence of Typhoid Fever in Relation to Age/Sex by Widal Test

Out of the 150 blood samples screened for Salmonella spp by Widal test, 38 were positive among the males, with the highest rate of infection observed among ages 27–37, while among the females, 25 were infected with the highest recorded among ages 16–26. There was a significant relationship ($\chi^2 = \text{Cal. 5.01} < \text{Tab. 11.07, df = 5}$) between the male and female categories (Table I).

3.2. Prevalence of Typhoid Fever in Relation to Occupation by Widal Test

Among the occupational groups, infection was more observed among students 32 (49.2%) and the least among the unemployed 4 (21%). Though there was, however, a significant relationship ($\chi^2 = \text{Cal. 8.68} > \text{Tab. 8.49, df = 1}$) between occupation and typhoid fever (Table II).
3.3. Prevalence of Typhoid Fever in Relation to Age/Sex by Blood Culture Test

The culture test revealed that 27 males were positive and were between the ages of 27–37. The same age group also recorded the highest infection rate, while 24 females were infected and were between the ages 16–26 and 27–37. There was, however, a co-relationship ($\chi^2 = \text{Cal. 5.46} < \text{Tab. 11.07, df} = 5$) between the sex and ages of the patients (Table III).

### TABLE III: Prevalence of Typhoid Fever in Relation to Gender by Widal Test

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Total no. examined</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>No. infected</td>
<td>No. examined</td>
</tr>
<tr>
<td>5–15</td>
<td>18</td>
<td>14</td>
<td>2 (14.2)</td>
</tr>
<tr>
<td>16–26</td>
<td>44</td>
<td>17</td>
<td>9 (52.9)</td>
</tr>
<tr>
<td>27–37</td>
<td>45</td>
<td>24</td>
<td>12 (50)</td>
</tr>
<tr>
<td>38–48</td>
<td>25</td>
<td>10</td>
<td>2 (20)</td>
</tr>
<tr>
<td>49–59</td>
<td>16</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td>60+</td>
<td>2</td>
<td>2</td>
<td>1 (50)</td>
</tr>
</tbody>
</table>

Note: $\chi^2 = \text{Cal. 5.46} < \text{Tab. 11.07, df} = 5$

3.4. Prevalence of Typhoid Fever in Relation to Occupation by Blood Culture Test

Among the occupational group, the culture test result showed that 51 were infected, with students recording the highest number of infections while the unemployed were the lowest. There was no significant relationship ($\chi^2 = \text{Cal.4.20 < Tab. 9.49, df} = 4$) between occupation and typhoid fever (Table IV).

### TABLE IV: Prevalence of Typhoid Fever in Relation to Occupation by Blood Culture Test

<table>
<thead>
<tr>
<th>Occupation</th>
<th>No. examined</th>
<th>No. infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student</td>
<td>65</td>
<td>31 (52.3)</td>
</tr>
<tr>
<td>Artisan</td>
<td>22</td>
<td>5 (22.7)</td>
</tr>
<tr>
<td>C. Servant</td>
<td>25</td>
<td>11 (72)</td>
</tr>
<tr>
<td>Trading</td>
<td>19</td>
<td>3 (26.3)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>19</td>
<td>1 (5.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>150</strong></td>
<td><strong>51</strong></td>
</tr>
</tbody>
</table>

Note: $\chi^2 = \text{Cal.4.20 < Tab. 9.49, df} = 4$

3.5. Determination of Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value

A two-by-two ($2 \times 2$) diagnostic table was used to carry out the calculation to determine the sensitivity and specificity of the widal and blood culture test using ELISA as a gold standard. The formula below was used:

3.5.1. Sensitivity (Positive for Disease)

$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} = \frac{\text{TP}}{\text{TP} + \text{FP}}$ (true positive)/TP + FN (true positive + false negative) = Probability of being tested positive when disease is present.

3.5.2. Specificity (Negative for Disease)

$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FN}} = \frac{\text{TN}}{\text{TN} + \text{FP}}$ (true negative)/TN = FN (true negative + false negative) = Probability of being tested negative when disease is absent.

3.5.3. Positive Predictive Value (PPV)

$\text{PPV} = \frac{\text{TP}}{\text{TP} + \text{FP}}$

PPV = TP (true positive)/TP + FP (true positive + false positive) = Probability of patient having disease when test is positive.

3.5.4. Negative Predictive Value (NPV)

$\text{NPV} = \frac{\text{TN}}{\text{TN} + \text{FN}} = \frac{\text{TN}}{\text{TN} + \text{FP}}$ (true negative)/FN + TN (false negative + true negative) = Probability of patient not having the disease when the test is negative.

3.6. Sensitivity, Specificity, PPV, and NPV of Widal and Blood Culture Test (ELISA as the Gold Standard)

The overall number of positive cases using the ELISA testing technique was 82. Widal test revealed 63 positive cases. Using ELISA as the gold standard, the following results were obtained: TP = 63, FP = 19, FN = 12, and TN = 56, while the sensitivity and specificity were 84% and 88.2%, whereas PPV and NPV were 84% and 82.3%, respectively (Table V).

On the other hand, the blood culture test showed 51 positive cases. Using ELISA as the gold standard, the following results were obtained: TP = 51, FP = 31, FN = 24, and TN = 44, while the sensitivity and specificity were 68% and 64.7%, whereas PPV and NPV were 62.1% and 64.7%, respectively (Table VI).

4. Discussion

The prevalence of typhoid fever by Widal test was higher among the males, 58.3%, and within the ages 27–37. It was lowest among the females, 37.0%, and among ages 16–26. This finding corresponds with the study by [16].
who observed the prevalence of typhoid fever to be 29.4% among males and 22.9% in females. Males might have acquired the infection due to high patronage of contaminated food vendors. At the same time, female counterparts are more concerned about their hygiene than males, thus increasing the frequency of typhoid fever (Table I). Among the occupational groups, infection was prevalent among students at 49.2% as compared to other occupations. The distribution of typhoid fever among the study population could be a result of their respective occupation since most of the patients were students, and more so, students usually patronize food vendors where the food might have been exposed to pathogens (Table II). The culture test showed 27 males had positive cases up to 50% within the ages 27–37, while 24 females were infected among the age group 16–26 and 27–37, showing high rates of infection, 66.6% and 57.1%, respectively. This result is in agreement with the findings of [17] in Abakaliki, [3] in Owerri, and [18] in Ondo, Nigeria, who observed 21.20%, 42%, and 73.3%, respectively. The reason for this high rate of infection could be because most patients in the study area resort to self-medication whenever they feel feverish; hence, the immune system becomes susceptible to Salmonella typhi parasite infection (Table III). In the occupational group, the culture test result showed that 51 were infected, with students recording the highest number of infections while the unemployed were the least.

From the ELISA (IgG) result, 82 (54.6%) were positive compared to Widal and blood culture test techniques. The sensitivity and specificity values for the Widal test were 84% and 88.2%, while PPV and NPV were 84% and 82.3% compared to (gold standard) ELISA, which showed 100% accuracy. The sensitivity and specificity values for the blood culture test were 68% and 64.7%, and PPV and NPV were 62.1% and 64.7% compared to (the gold standard). The result of this study is in conformity with a study carried out by [19] where sensitivity and specificity values were 93% and 96%, respectively. Also, similar research conducted among 100 patients diagnosed with typhoid fever by Widal test, blood culture, and ELISA revealed a sensitivity, specificity, positive and negative predictive values of 63.6%, 79.4%, 46.6%, and 88.55% [20]. In contrast, ELISA’s sensitivity, specificity, and positive and negative predictive values were 95.4%, 80.7%, 58.3%, and 98.4%. In comparing the Widal and blood culture test to the ELISA testing technique, ELISA demonstrated higher accuracy than the Widal and blood culture test. Even though blood culture remains the gold standard for diagnosing typhoid fever, the ELISA testing technique should be adopted since it shows high accuracy.

5. Conclusion

Even though typhoid fever is common in Nasarawa state and has a similar characteristic to malaria, most of the patients sampled in the study area were infected with typhoid among the study population. In conclusion, this study demonstrates that specific diagnoses are important in managing and controlling infectious diseases. The multiplicity of infection among typhoid patients was high, indicating a very high transmission rate due to the prevalence of polyclonal infection. Based on this result, typhoid diagnosis using Widal diagnostics is still a sub-optimal option due to the possibility of a false positive result. Though the Widal test result gave a relatively true positive result compared to the ELISA test, the gold standard should be molecular assay (ELISA or PCR), which is more sensitive and specific.

Acknowledgment

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

References


